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VECTOR SPECIFICITY IN POTYVIRUS TRANSMISSION: ROLE OF THE HELPER COMPONENT

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I. Overall Summary and Statistics:

Abstract

Objectives: The overall objective of this research was to gain a better understanding of how potyviruses interact with their aphid vectors. The aim was to design new approaches for prevention of potyvirus spread by aphids. The sub-objectives included: (1). Determination of which of the HCs of different potyviruses effect efficient transmission by specific aphid vectors; (2). Determine regions in the HC that play a role in their compatibility with the vector; (3). Determine the factors within the aphid stylets that modify HC activity in transmission.

Background of the topic: Background to the topic: Potyviruses are typical non persistent viruses. They are retained within the vector's stylets and rapidly lost by the vector. Some potyviruses greatly differ in their ability to be transmitted by different aphid species. The present work centered on analyzing factors that may modify the interactions between the "helper component"(HC), the virions and the aphid species involved.

Major conclusions, solutions and achievements: It was established that specificity of transmission may depend on aphid species used. It was also shown that specificity may depend on the affinity between HC and virion. However, the attempts to create **active** chimeric TEV/TuMV HCs or ZYMV/TuMV HCs to identify the regions that determine interaction with a specific vector(s), were not successful.

More progress was attained in objective 3: In Kentucky, tests were conducted to ascertain retention tobacco vein mottling virus (TVMV) HC in the stylets of *L. erysimi* compared to that in *M. persicae*. Ultra-thin section of stylets of aphids that fed on either TuMV HC or TVMV HC antibodies were treated with gold-labeled goat anti-rabbit antibodies. TuMV was seen in 25% the stylets of *L. erysimi* when they acquired TuMV HC but not when they acquired TVMV HC. In *M. persicae*, TVMV HC was present in 30% of the stylets. . Transmission with TuMV HC was not affected by treatment with *L. erysimi* saliva whereas transmission with PVY HC (which also is not functional in L. erysimi) was consistently reduced by about half. Saliva from *M. persicae* had essentially no effect on either HC.

The possible role aphid cuticle proteins (which are found on the stylets surface) in the association with the potyviral HC was investigated in Israel. This was done adopting two approaches: (a) isolation of cuticular proteins from aphid cuticle; (b) screening for genes encoding cuticular proteins. In the first approach, we succeeded in extracting proteins from whole homogenized *M. persicae* using concentrated urea. The extracted protein served for preparation of anti cuticular antibodies. In overlay experiments it was found that cuticular proteins specifically bind to ZYMV HC. In addition, a cDNA library of *M. persicae* has been prepared. Genes encoding for cuticular proteins were ascertained using antibodies to cuticular proteins. This allowed reporting the sequence of the first cuticular gene of aphids and comparing it in six aphid species.

Implications, scientific and agricultural: Achievements: (1) Proofs were provided for the role of the specificity of the aphid species to the HC of certain potyviruses; (2) aphid's saliva was found to affects transmission efficiency; (3) cuticle protein genes were isolated for the first time from aphid species and an association of cuticle protein with the potyviral HC was discerned.

Agricultural and/or economic impact of the research findings: At this stage of research, our finding do not bear an agricultural or economic impact.

Details of cooperation: Cooperation between the two laboratories enabled comparisons of results and overcoming problems encountered in the two laboratories. . In 2002, B. Raccah visited Lexington for research efforts coordination.

C. Achievements

Significance of the main achievements and innovations:

Objective 1: The intent of this objective 1 was to test additional HC-virus combinations to determine the extent of HC specificity. The experiments to test the hypothesis that the ability of a species to use a specific HC is correlated with the adaptation of the virus and the aphid to a common host plant. The level of funding was reduced relative to that originally proposed. Therefore, in Kentucky, we concentrated our efforts on identifying features in the helper component (HC) and in aphids that are responsible for vector specificity. To do this, we used the differential system involving *Myzus persicae*, which is able to transmit both Tobacco Etch Virus (TEV) and Turnip Mosaic Virus (TuMV) and *Lipaphis erysimi*, which is able to transmit TuMV but not TEV from infected plants. In Bet Dagan, a similar approach was adopted, where *M. persicae* and *Aphis gossypii* transmit both zucchini yellow mosaic virus (ZYMV) and TuMV and *L. erysimi* and *Brevicorynae brassicae* were do not transmit ZYMV.

Since previous work had shown that *L. erysimi* could transmit TEV from an *in vitro* system if supplied with TuMV HC, it was evident that HC was controlling transmission and we proposed to identify the domains in TEV HC and TuMV that differed and hence accounted for differential transmission. To do this, we used a number of strategies. Initially, we attempted to use a variety of systems to express HC *in vitro*. Although we were able to express products that seemed to be authentic HC, based on gel analyses, none of these products were functional in transmission. Our next strategy was to create chimeric viruses that incorporated TuMV HC or parts thereof into full-length clones of TEV. We were successful in producing a chimera that substituted the entire TuMV HC sequence for the TEV HC sequence in a full-length TEV clone. These HCs are very similar in size, conserved domains and proteinase cutting sites. To create the chimera, the infectious plasmid construct pTEV-HCHXa, which contains a His-tag followed by an Mlu I cutting site at the 5' end of the HC was modified by introducing a Sac II cutting site at the 3' end of the HC. A TuMV construct was modified by introducing flanking Mlu and Sac II sites by PCR. Mechanical inoculation showed that the clone was infectious in both peppers (host of TEV) and mustard (host of TuMV but not TEV). The chimeric virus was transmitted readily from plants by *M. persicae*, and, according to the hypothesis, should have been transmissible by *L. erysimi*, but disappointingly it was not. Attempts to obtain purified TuMV HC from chimera-infected plants yielded only small amounts of HC, which did not even support virus transmission by *M. persicae*. One explanation could be that the presence of the his-tag on the TuMV HC somehow interferes with the

functioning of the HC in *L. erysimi*. The fact that the substitution of TuMV HC for TEV HC allowed the chimera to infect peppers and to alter the symptom phenotype on tobacco demonstrates that the virus-spread function of the HC was active, although the vector specificity function was not.

Objective 2. The second area of concentration was testing the hypothesis that inability of *L. erysimi* to utilize Tobacco vein mottling virus (TVMV) HC was due to inability of the HC to be retained by the stylets.

Our primary efforts are being directed toward determining whether the inability of *L. erysimi* to utilize TVMV HC is due to an inability of this HC to be retained by the stylets. To do this, *L. erysimi* and *M. persicae* were allowed to acquire either TuMV HC or TVMV HC. The insects were immediately killed with CO_2, and the heads removed , fixed and embedded. The stylets were then ultra-thin sectioned, and the sections probed with either TuMV HC or TVMV HC antibodies and then treated with gold-labeled goat anti-rabbit antibodies. Approximately 50 sections from each aphid's stylets were examined. Three separate experiments were performed and the hypothesis was supported: in one positive control, 25% (10/40) *L. erysimi*contained TuMV in the stylets, whereas 0% (0/40) contained TVMV HC. In the other positive control, *M. persicae*, TVMV HC was present in 30% (9/30) of the stylets.

Objective 3. One possibility for these results could be differential effects of saliva on respective HCs. As a model, we tested the effect of saliva from *M. persicae* and *L. erysimi* on TuMV HC and PVY HC (which also is not functional in L. erysimi). Saliva from the respective aphids was collected , mixed with the respective HCs and with TVMV virions , and fed to *M. persicae* to test for virus transmission. Control HCs were not treated with saliva. Transmission with TuMV HC was not affected by treatment with *L. erysimi* saliva (85% for treated and 87% for control), whereas transmission with PVY HC was consistently reduced by about half (average 56% to 31%). Saliva from *M. persicae* had essentially no effect on either HC, 84% vs 78% transmission with TuMV HC and 63 vs 65% transmission with PVY HC.

In attempts to find a site in the stylet where the HC may interact, in Bet Dagan, we isolated the cuticular protein (CuP) genes from several aphids (differing in potyvirus transmissibility). The first gene was obtained by building a cDNA library from the aphid *Myzus persicae* and screening its expressed proteins with polyclonal antibodies raised against a lepidopteran CuP. One of the clones whose sequence corresponds to a "hard" CuP partial gene was selected. The full-length gene (*Mpcp1*) was reconstructed by further screening of the cDNA library, completed by 5'RACE from purified total RNA of aphids. Comparison of the gene sequence

with available sequences revealed that it is a new cuticle protein gene, with special features. The deduced amino-acid sequence suggested a putative signal peptide. The mature protein is composed of a central mostly hydrophilic domain that corresponds to the chitin-binding extended R&R domain, which is surrounded by quasi-hydrophobic N- and C-terminal domains, rich in alanine, tyrosine and regularly dispersed proline. The C-terminal domain is constituted uniquely of a stretch of a repeat of 14 conserved and 4 altered (A/S/K)APAY motives. The mRNA of *Mpcp1* could be detected in three larval stages of the aphid. Primers respective to sequences flanking the CuP coding region were used to isolate cuticle protein genes from five other aphid species (*Lipaphis erysimi, Aphis gossypii, Brevicoryne brassicae, Rhopalosiphum maidis,* and *Aphis fabae*). The sequence of the deduced cuticle proteins from those species was compared. The comparison revealed a high degree of similarity. Other genes were cloned using primers specific to hard cuticular gene. Surprisingly, they were found to be closely related to *Mpcp1*, differing in the number of repeats. These data suggest that this CuPs family is specific to aphids and seems to represent a predominant structural element of its integument.

Cuticle protein extraction: In order to study a possible interaction between CuP and a potyviral HC, the CuP was extracted from frozen aphids. Aphids were treated with Tris buffer, phenylmethyl-sulfonyl fluorid (PMSF) and β-mercaptoethanol(Chtin extraction buffer). The outcome was subjected to a mixture of Urea and PMSF mixture. The supernate that was dialyzed overnight served for protein detection. Visualization of CuP was made after separation on an SDS polyacrylamide electrophresis (PAGE) using either Coomassie staining or and silver staining. Immunoassay (Western blots) has been made possible upon the receipt of a anti *Manduca sexta* cuticular protein serum (kindly provided by L. Riddiford, Washington University, USA).

The protein bands reacting to the anti CuP from lepidoptera served for preparation of antibodies to aphid CuP. A strong and specific reaction has been obtained to aphid CuP blotted into nitrocellulose membrane.

Binding assay of cuticle protein to the HC-Pro: After separation on SDS-PAGE, the *M. persicae* cuticular proteins were transferred to nitrocellulose membranes. The membranes were blocked 4 hours at 4˚C with chitin binding buffer mixed with milk powder. Then the membranes were soaked overnight at 4˚C with chitin binding buffer containing purified HC or

infected plants the HC contain 6 residue of the amino acid Histidine. Detection for the HC bound to the cuticle protein was made using a monoclonal Abά His tagged and discerned using an anti mouse alkaline phosphates.